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Serum and lymphocyte levels of heat shock protein 70 in aging: a study in the normal Chinese population

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Abstract Heat shock proteins (Hsps) have been reported to play an important role in both physiological and pathological processes. Hsps also may serve as biomarkers for evaluating disease states and exposure to environmental stresses. Whether Hsp levels in serum and lymphocytes are correlated with age and sex is largely unknown. In this study, we analyzed serum Hsp70 (the most abundant mammalian Hsp) levels by using Western dot blot in 327 healthy male donors aged between 15 and 50 years. We also investigated the association between Hsp70 levels and age in lymphocytes of 80 normal individuals aged between 40 and 77 years because various chronic diseases increase after the age of 40 years. Our data showed that serum Hsp70 levels were positively correlated with age in subjects aged between 15 and 30 years ($P < 0.05$) but negatively correlated with age in subjects aged between 30 and 50 years ($P < 0.05$). Serum Hsp70 levels were the highest in individuals aged between 25 and 30 years among all age groups. In the lymphocyte study there also was a significant age-related decrease in Hsp70 levels in lymphocytes of individuals older than 40 years. The Hsp70 levels were negatively correlated with age ($r = -3.708$, $P < 0.0001$) but not with sex ($r = -10.536$, $P = 0.452$). This suggests that both serum and lymphocyte Hsp70 levels are age-related and that these may be linked to age-related stress. Thus, age is an important factor in using serum and lymphocyte Hsp70 as biomarkers to evaluate the disease states or exposure to environmental stresses (or both).

INTRODUCTION

All organisms share a conserved stress response that involves an induced synthesis of heat shock or stress proteins (Hsps) when they are exposed to elevated temperature and to other environmental challenges (Reviewed in Lindquist and Craig 1988; Morimoto et al 1994). Hsps can be grouped into 4 general families (Hsp90–110, Hsp/heat shock cognate 70 [Hsc70], Hsp60, and the small Hsps [Hsp10–30]) on the basis of their apparent molecular masses. The best-known Hsp is the inducible member of the Hsp/Hsc70 family, with an apparent molecular mass of 71 and 72 kDa in rat and humans, respectively, and referred to in this study as Hsp70. Hsps are involved in numerous functions and can protect against cell damage (Hightower 1991; Parsell and Lindquist 1994). Hsps of the Hsp/Hsc70, Hsp60, and Hsp90 families also have been shown to function as molecular chaperones, facilitating the synthesis, folding, assembly, and intracellular transport of many proteins (Gething 1992). Therefore, it is not surprising that Hsps have been found to be implicated in physiological (eg, development and aging) and pathological (eg, fever, infection, ischemia) processes (reviewed in Welch 1992; Minowada and Welch 1995; Zugel

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and Kaufmann 1999). Hsps also may serve as biomarkers for evaluating disease states (Wright et al 2000) and damages resulting from exposure to environmental stresses (Xiao et al 2002, 2003).

In humans, aging is often associated with an increased incidence of infections and general morbidity and mortality (Jones et al 1982; Lithgow and Kirkwood 1996), and age and sex are important confounding factors in evaluating diseases. Studies in cultured cells and in animal models have demonstrated that the stress response is age dependent (Fargnoli et al 1990; Blake et al 1991; Heydari et al 1994; Lithgow et al 1995; Kregel and Moseley 1996; Locke and Tanguay 1996; Locke 2000). An age-related decrease in major Hsps also has been reported in human peripheral blood cells (Rao et al 1999, 2003; Rea et al 2001; Njemini et al 2002). In humans, an aberrant expression of Hsps has been linked to disease states and might be of significance in the pathogenesis and prognosis of diseases (reviewed in Welch 1992; Minowada and Welch 1995). Few studies have been conducted on the measurements of Hsp70 levels in the normal population. Such studies are important to provide basic data for comparison of Hsp70 levels in normal human populations with those observed in stressed or diseased populations. In this study, we investigated whether there was an age-related change in serum Hsp70 levels in 327 healthy male volunteers aged between 15 and 50 years. We also determined the level of Hsp70 in lymphocytes of individuals aged between 40 and 77 years and analyzed the correlation of Hsp70 levels with age and sex by linear regression analysis.

MATERIALS AND METHODS

Subjects

After obtaining oral informed consent, the 327 healthy male volunteers who were aged between 15 and 50 years and lived in Wuhan, Central China, had a complete physical examination by doctors at both Union Hospital and Tongji Hospital affiliated to Tongji Medical College and at Wugang Hospital affiliated to The Wuhan Steel Company. This complete annual checkup consisted of a questionnaire comprising 47 items ranging from medical history, life style (smoking, alcohol consumption, food regime), full physical examination (weight, height, signs, blood pressure, heart rate, oral temperature, electrocardiogram, chest X-ray etc) to laboratory tests (blood and urine tests, glucose, blood fat, hepatitis antigens, and antibodies). Each subject donated a 1-time blood sample for determining serum Hsp70 levels. The subjects were divided into 7 age groups of 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, and 45–50 years.

Another group of 80 apparently healthy subjects living in Kunming city (Yunnan Province) also were recruited at Yanan Hospital, a community hospital in the area, for detection of lymphocyte Hsp70 levels. These 80 subjects comprised 42 men and 38 women aged between 40 and 77 years and were divided into 3 age groups: 40–49 years (n = 28; 16 men and 12 women), 50–59 years (n = 26; 12 men and 14 women), and >60 years (n = 26; 14 men and 12 women).

All subjects were physically well and did not have any active diseases such as hypertension, coronary heart disease, nervous and psychological diseases, cancer, and other acute and chronic diseases. The groups were similar in their living and working environments as well as in their use of tobacco and alcohol. Any subject who had abnormal values in any of these tests was excluded from the present study. So, all subjects included are considered normal on the basis of all these criteria. The study was approved by the Ethics Committees of Tongji Medical College, Wugang Hospital, and Yanan Hospital.

Blood sampling

Venous blood was sampled in the morning after overnight fasting. For the 327 male subjects from Wuhan, 1 mL of venous blood was collected in a tube without heparin. Approximately 5 mL of venous blood was collected from the 80 subjects of Kunming into a heparinized tube and used to isolate lymphocytes using Ficoll-Hypaque (Biochemical Reagent Co, Shanghai, China), as described earlier (Xiao et al 2003). The cell viability was >95%, and cell counts were within normal range for all groups. The collected lymphocytes were washed twice with phosphate-buffered saline (PBS), counted, and the number of lymphocytes was adjusted to 5000/μL with PBS. Two hundred microliters of lymphocytes in PBS was centrifuged at room temperature and the buffer solution removed as soon as possible to avoid induction of Hsps during handling. Sera and lymphocytes were stored at −80°C for further analysis.

Detection of Hsp70 in serum

Serum Hsp70 level was determined using Western dot blot. Briefly, 20 μL of serum at a 1:20 dilution was loaded by pipetting on a small piece of precut nitrocellulose membrane using a vacuum system. The load was monitored by staining with Ponceau S (Sigma, St. Louis, MO, USA). Membranes were then saturated with blocking buffer (PBS containing 5% skim milk powder) for 1 hour at 37°C with gentle agitation and washed with PBS-0.05% Tween 80 for 5 minutes. A rabbit anti-human Hsp70 antibody specific for the inducible Hsp71 (#799 in Tanguay et al 1993) was added at a dilution of 1:1000 in PBS containing 5% skim milk powder and the membranes incu-

bated at 37°C for 1 hour with gentle agitation. After washing membranes 6 times (10 minutes each time) with 200 mL PBS-0.05% Tween 80, horse radish peroxidase (HRP)–labeled goat anti-rabbit immunoglobulin G (IgG) in blocking buffer (1:1000) was added and membranes incubated at 37°C for another hour. Membranes were washed 4 times with 200 mL PBS-0.05% Tween 80. The presence of Hsp70 was revealed using 3,3-diaminobenzidine tetrahydrochloride buffer (DAB) for 3–5 minutes, as described previously (Wu et al 2001b). The levels of Hsp70 were quantified using an imaging densitometer (Shimadzu CS-930, Shimadzu, Japan) at 460 nm, and the value presented is the integrated optical density or the value in nanograms per milliliter. For the latter, purified recombinant human Hsp70, obtained through the expression of the human complementary deoxyribonucleic acid (cDNA) coding for the inducible Hsp71 in NaCl-induced *Escherichia coli* GJ1168 cells using pET30 as expression vector (Tanguay et al 1993; Bhandari and Gowrishnkar 1997), was used as the standard at the following concentrations: 0.01, 0.05, 0.10, 0.20, 0.30, and 0.40 μg/mL. Hsp70 level was calculated from this standard curve in nanograms per milliliter.

Determination of Hsp70 in lymphocytes

Determination of Hsp70 level in lymphocytes was performed as described previously (Xiao et al 2002) with minor modifications. Briefly, the collected cells were mixed with 1× sodium dodecyl sulfate (SDS) sample buffer, boiled at 100°C for 5 minutes, and loaded onto SDS-polyacrylamide gel. Equal numbers of cells from each subject were loaded in individual lanes. After electrophoresis, proteins were transferred electrophoretically to nitrocellulose membranes. The membrane was saturated with blocking buffer (PBS containing 5% skim milk powder) for 1 hour at 37°C with gentle agitation and washed with PBS-0.05% Tween 80 for 5 minutes. It was then incubated at 37°C for 1 hour with gentle agitation with rabbit anti-Hsp70 antibodies diluted 1:1000 in PBS containing 5% skim milk powder. After washing the membranes 6 times (6 × 10 minutes) with 10 mL PBS-0.05% Tween 80, HRP-labeled goat anti-rabbit IgG in blocking buffer (1:1000) was added and the membrane further incubated at 37°C for another 1 hour and washed 6 times with 10 mL PBS-0.05% Tween 80. Hsp70 was revealed using DAB buffer for 3–5 minutes, and Hsp70 was quantified using an imaging densitometer (Shimadzu CS-930, Shimadzu) and the integrated optical density used to represent the relative levels of Hsp70.

Statistical analyses

All data were analyzed using SPSS and presented as mean ± SD using Student's *t*-test. Other analyses were carried out using analysis of variance (ANOVA) and multivariate linear regression models with an *F* test. Statistical inferences are based on the levels of significance ($P < 0.05$).

RESULTS

Serum Hsp70 levels vary with age

Circulating Hsps and corresponding antibodies have been found previously in sera of normal individuals (Pockley et al 1998, 1999; Wright et al 2000). Serum Hsps and their antibodies have been used as biomarkers for diseases and as markers of environmental stress (Wu et al 1998, 2001a, 2001b; Xu et al 1999; Zugel and Kaufmann 1999; Pittet et al 2002). Therefore, we determined serum Hsp70 levels of healthy male volunteers aged between 15 and 50 years. Table 1 lists the concentration of serum Hsp70 in the different age groups. Serum Hsp70 levels increased in subjects aged between 15 and 30 years ($P < 0.05$), peaked between 30 and 39 years, and were lower in the 40- to 50-year group. Hsp70 value was highest in the group of individuals aged between 25 and 30 years and lowest in individuals aged between 45 and 50 years ($P < 0.05$). There was a slight decrease in serum Hsp70 in the 30- to 34-year and 35- to 39-year age groups as compared with the 25- to 29-year age group, but the difference was statistically significant ($P < 0.05$). Figure 1 shows a graphic representation of the serum Hsp70 level for all individuals. As can be seen, there is a large variation between levels of Hsp70 in individuals. The variation is less pronounced in aged subjects.

Lymphocyte Hsp70 levels decrease during aging

Human lymphocytes are the common surrogate for investigating the biomedical significance of many genes and proteins in studies of diseases and response to environmental stresses (Bonassi and Au 2002). The expression level of Hsp70 in these cells may be an important criterion in studying human stress. We therefore determined Hsp70 levels of lymphocytes in a different population of 80 subjects (42 men, 38 women) aged between 40 and 77 years and divided into 3 age groups. The values of lymphocyte Hsp70 in the 3 different age groups are summarized in Table 2 and the values for individuals plotted in Figure 2. The Hsp70 level of lymphocytes was higher in individuals in the 40- to 49-year group, decreased in the 50- to 54-year group, and was the lowest in the group of subjects over 60 years of age ($P < 0.05$). Thus, there is a significant decrease in Hsp70 level as age increases ($P < 0.05$). Figure 2 shows the individual values of Hsp70 in each subject aged between 40 and 77 years. Although there is a variation in Hsp70 level in lympho-

Table 1 Serum Hsp70 levels in different age groups of Chinese normal subjects

	Age group (y)						
	15–19	20–24	25–29	30–34	35–39	40–44	45–50
Number of individuals	57	27	74	42	22	48	57
Hsp70 level (mean ± SD) (ng/mL)	97.20 ± 26.54*	103.97 ± 25.40	118.16 ± 27.61	116.77 ± 37.37	116.79 ± 24.24	87.90 ± 23.44*	85.15 ± 19.52*

Hsp70, heat shock protein 70.
* $P < 0.05$; compared with 20-, 25-, 30-, 35-age groups.

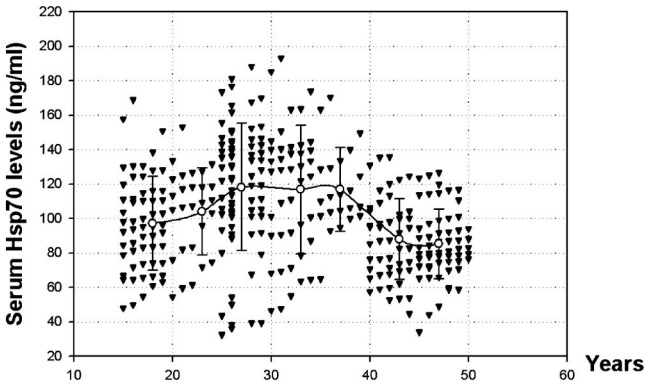

Fig 1. Levels of serum heat shock protein 70 (Hsp70) in all individuals of the 7 age groups of donors. The mean for each age group is plotted in the center of the graph. The Hsp70 values are in nanograms per milliliter of serum.

Table 2 Hsp70 level of lymphocytes in different age groups

Age groups (y)	Number of samples	Hsp70 level (mean ± SD integrated optical density)
40–49	28	198.11 ± 77.72*
50–59	26	130.33 ± 55.70*
>60	26	89.38 ± 30.73*

ANOVA, analysis of variance; Hsp70, heat shock protein 70.
* $P < 0.05$; compared among 3 groups (F test of ANOVA).

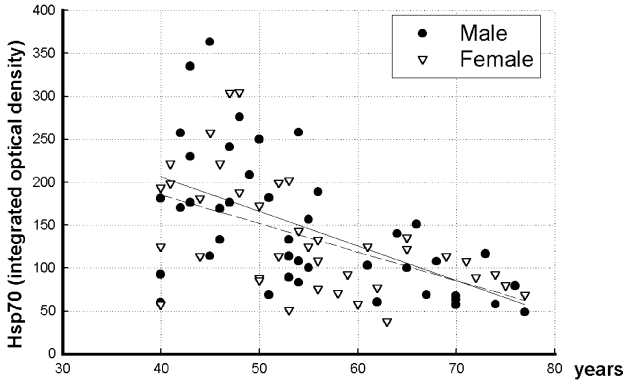

Fig 2. Variation in lymphocyte heat shock protein 70 (Hsp70) levels as a function of age. Lymphocytes were isolated from 80 subjects aged between 40 and 77 years, and Hsp70 levels were determined by Western blot. The levels of Hsp70 are given in IOD. IOD, integrated optical density.

cytes of individual among the same age group and between different age groups, there is a clear tendency for decrease in lymphocyte Hsp70 in aged individuals. The largest variation (about 7-fold) was observed in the youngest age group, whereas the smallest variation (about 2-fold) was observed in the oldest group.

No difference in these variations was observed between sexes. We also examined whether there was any correlation of Hsp70 levels in lymphocytes of these individuals with age and sex using the model of multivariate linear

Table 3 Association between Hsp 70 and sex and age by multivariate linear regression analysis

Variables	Partial regression coefficient	Standardized partial regression coefficient	*t*-test	*P* value
Model 1				
Constant	360.2025			
Sex	−10.5360	−0.0720	−0.76	0.4522
Age	−3.7076	−0.5443	−5.72	<0.0001
Model 2				
Constant	344.7876			
Age	−3.7099	−0.5446	−5.74	<0.0001

Hsp70, heat shock protein.

regression to further understand a possible role of aging. Such data also can be of value when Hsp70 is used as a biomarker in evaluation of disease states and exposure to environmental stresses. Table 3 lists the correlations of Hsp70 with sex and age. As shown, there was a significant negative correlation of Hsp70 levels with age ($P < 0.0001$), but no significant correlation of this protein with sex was observed ($P = 0.4522$). Further analysis of these data was performed using a linear regression model built with a forward stepwise selection procedure (the P value for entry and removal, 0.10 and 0.15, respectively) and the results also are presented in Table 3. This model excluded sex as a predictor. It is apparent that there still was a significant negative correlation between Hsp70 level and age in these individuals ($r = -3.7076$, $P < 0.0001$). The best regression model shows as Y (Hsp70) = 344.7876 − 3.7099X (age in years). Thus, age is a clear predictor of lymphocyte Hsp70 levels.

DISCUSSION

Human aging is characterized by a progressive, intrinsic, and generalized imbalance of control of many regulatory systems (Finch and Tanzi 1997; Pawelec et al 1997; Masoro and Austad 2001). Aging is accompanied by a decay of self-defensive mechanisms and by an accumulation of damages at the molecular, cellular, and organismal level as a result of a constant exposure to adverse environmental stresses (Sherman and Goldberg 2001; Söti and Csermely 2002). These stresses trigger the stress response, resulting in the synthesis of Hsps that play an important role in cytoprotection. Hsps are thus particularly important in physiological and pathological processes and also can serve as biomarkers to evaluate the extent of disease or the degree of environmental stresses. Studies using cultured cells and animal models suggest that Hsps play a key role in aging and longevity (Heydari et al 1994; Lithgow et al 1995; Kregel and Moseley 1996). Rao et al (1999) showed that in humans there was an age-related attenuation in the expression of major Hsps by comparing the expression of lymphocytes from 8 young donors aged between 16 and 29 years and from 4 older individuals aged between 76 and 84 years. A subsequent study using cDNA microarray analysis (Rao et al 2003) suggested that there were age-related differences in the global expression profile as well as in the translational kinetics of Hsps in the lymphocytes from 15 healthy young donors (aged between 19 and 29 years) and 10 old donors (aged between 71 and 85 years). Similar results and conclusions were reported by Rea et al (2001) in a study of 60 individuals and by Njemini et al (2002) in a study of 19 young (aged between 21 and 38 years), 21 middle-aged (between 40 and 64 years), and 10 older (between 70 and 81 years) individuals.

In our large study of 327 healthy normal Chinese subjects from central China, we found an age-related variation in serum Hsp70 levels, with a clear decrease in subjects over 40 years of age. Our data show that there is a peak in the Hsp70 levels in those individuals whose ages are between 25 and 40 years ($P < 0.05$, compared with other groups). These data are consistent with those of Rea et al (2001) who studied 60 individuals aged between 20 and 96 years.

The biological relevance of these age-related changes in Hsp70 levels in normal individuals is presently unknown and needs further investigation. However, we speculate that Hsp70 may be a biomarker for aging because some of the strongest candidate genes to influence aging and longevity are genes that regulate the processes of somatic maintenance and repair, such as the stress-response system (including the inducible Hsp70) (Lithgow and Kirkwood 1996). Hsp70 also plays a key role in protection against various stresses (Angelidis et al 1991; Li et al 1991; Marber et al 1995; Plumier et al 1995, 1997) and functions as a molecular chaperone, facilitating the folding and repair of misfolded and damaged proteins resulting from aging (Muchowski et al 2000; Nardai et al 2002). Some studies suggested that Hsps play a role in modifying the immune response, which is related to the ability to respond to various stresses and aging processes (Zugel and Kaufmann 1999; Basu and Srivastava 2000; Cappisi and Fleshner 2002; Prohaszka et al 2002). Finally, several reports suggested that serum Hsp70 may be very important in normal physiological and stressful conditions. The exact mechanisms by which Hsp70 is released in serum are presently unknown. Hence, some Hsps may be released in cells experiencing stress, and these Hsps can be taken up by neighboring cells (Hightower and Guidon 1989). Serum Hsp also might come from apoptotic cells. Elevated serum level of Hsp70 has been associated with an enhanced survival in patients who had experienced severe trauma (Pittet et al 2002). It has been demonstrated that older patients with acute heat-induced illness exhibited lower Hsp70 levels than younger ones

(Wang et al 2001). Exercise increases serum Hsp70 (inducible one) released from other tissues or organs in humans, which suggests that Hsp70 may have a systemic role (Walsh et al 2001).

Our data on the Kunming subjects show that Hsp70 levels in lymphocytes also decrease with aging. There are other reports of aged-related changes in Hsp70 in human lymphocytes (Rao et al 1999). Njemini et al (2002) showed that lymphocyte Hsp70 was inversely related to the age of their subjects (aged between 20 and 80 years), but they did not elaborate on this finding. Further investigation on a correlation between serum Hsp70 and lymphocyte Hsp70 levels under normal and stressed conditions seems warranted.

There is an increase in the incidence of infections and in general morbidity and mortality in the elderly, and human lymphocytes are the common surrogate for investigating the biomedical significance of many genes and proteins in some environmental diseases and stresses. Our results further demonstrate that there is a significant decrease in normal and baseline Hsp70 levels of lymphocytes in individuals aged between 40 and 77 years as age increases, and there was a significant negative correlation of Hsp70 levels with age but not with sex. These results can be explained by Hsp70's possible function and roles, especially in its cytoprotection against various stresses (Hightower 1991; Muchowski et al 2000). Finally, our results showed that there was a variation in serum (Fig 1) and lymphocyte (Fig 2) Hsp70 levels among individuals of different ages and even of the same age, suggesting that both serum and lymphocyte Hsp70 levels may be related to age and aging. The largest variation observed in the youngest age group may result from more variable levels of physical activities and basal metabolism compared with the oldest age group. This larger variation in young subjects also can be observed in the data of Njemini et al (2002). Therefore, the normal reference values of serum and lymphocyte Hsp70 levels in individuals with different ages observed using a standard detection method are very important in using Hsp70 as a biomarker to evaluate disease states and environmental stresses. It has been suggested that there are variations in Hsp gene expression and DNA sequences in animals and humans, and these variations may contribute to differential disease susceptibility and level of stress tolerance (Favatier et al 1997; Xiao et al 2003). However, it still remains to be confirmed whether serum and lymphocyte basal Hsp70 levels are related to aging or longevity and in which ways.

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REFERENCES

Angelidis CE, Lazaridis I, Pagoulatos GN. 1991. Constitutive expression of heat shock protein 70 in mammalian cells confers thermotolerance. *Eur J Biochem* 199: 35–39.

Basu S, Srivastava PK. 2000. Heat shock proteins: the fountain-head of innate and adaptive immune response. *Cell Stress Chaperones* 5: 443–451.

Bhandari P, Gowrishnkar J. 1997. An Escherichia coli host strain useful for efficient overproduction of cloned gene products with NaCl as the inducer. *J Bacteriol* 179: 4403–4406.

Blake MJ, Udelsman R, Feulner GJ, Norton DD, Holbrook NJ. 1991. Stress-induced HSP70 expression in adrenal cortex: a glucocorticoid sensitive, age-dependent response. *Proc Natl Acad Sci U S A* 88: 9873–9877.

Bonassi S, Au WW. 2002. Biomarkers in molecular epidemiology studies for health risk prediction. *Mutat Res* 511: 73–86.

Cappisi J, Fleshner M. 2002. Role of extracellular HSP72 in acute stress-induced potentiation of innate immunity in active rats. *J Appl Physiol* 94: 43–52.

Fargnoli J, Kunisada T, Fornace AJ Jr, Schenider EL, Holbrook NJ. 1990. Decreased expression of heat shock protein 70mRNA and protein after heat treatment in cells of aged rats. *Proc Natl Acad Sci U S A* 87: 846–850.

Favatier F, Bornman L, Hightower LE, Gunther E, Polla BS. 1997. Variation in hsp gene expression and Hsp polymorphism: do they contribute to differential disease susceptibility and stress tolerance? *Cell Stress Chaperones* 2: 141–155.

Finch CE, Tanzi RE. 1997. Genetics of aging. *Science* 278: 407–411.

Gething MJ. 1992. Protein folding in the cell. *Nature* 355: 33–45.

Heydari AR, Takahashi R, Gutsmann A, You S, Richardson A. 1994. Hsp70 and aging. *Experientia* 50: 1092–1098.

Hightower LE. 1991. Heat shock, stress protein, chaperones, and proteotoxicity. *Cell* 66: 191–197.

Hightower LE, Guidon PT. 1989. Selective release from cultured mammalian cells of heat shock(stress) proteins that resemble glia-axon transfer proteins. *J Cell Physiol* 138: 257–266.

Jones TS, Liang AP, Kilbourne EM, et al. 1982. Morbidity and mortality associated with the July 1980 heat wave in St Louis and Kansas City, Mo. *JAMA* 247: 3327–3331.

Kregel KC, Moseley PL. 1996. Differential effects of exercise and heat stress on liver hsp70 accumulation with aging. *J Appl Physiol* 80: 547–551.

Li GC, Li LY, Liu K, Mak JK, Chen L, Lee WMF. 1991. Thermal response of rat fibroblasts stably transfected with the human

70kDa heat shock protein encoding gene. *Proc Natl Acad Sci U S A* 88: 1681–1685.

Lindquist S, Craig EA. 1988. The heat shock Proteins. *Ann Rev Genet* 22: 631–677.

Lithgow GJ, Kirkwood TB. 1996. Mechanisms and evolution of aging. *Science* 73: 80s.

Lithgow GJ, White T, Molov S, Johnson TE. 1995. Thermotolerance and extended life-span conferred by single-gene mutation and induced by thermal stress. *Proc Natl Acad Sci U S A* 92: 7540–7544.

Locke M. 2000. Heat shock transcription factor activation and hsp72 accumulation in aged skeletal muscle. *Cell Stress Chaperones* 5: 45–51.

Locke M, Tanguay RM. 1996. Diminished heat shock response in the aged myocardium. *Cell Stress Chaperones* 1: 251–260.

Marber MS, Mestril R, Chi S, Sayen MR, Yellon DM, Dillmann WH. 1995. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest* 96: 1446–1456.

Masoro EJ Austad SN, eds. 2001. *Handbook of the Biology of Aging.* Academic Press, San Diego.

Minowada G, Welch WJ. 1995. Clinical implications of the stress response. *J Clin Invest* 95: 3–12.

Morimoto RI, Tissières A, Georgopoulos C, eds. 1994. Progress and perspectives in the biology of heat shock proteins and molecular chaperones. In: *The Biology of Heat Shock Proteins and Molecular Chaperones.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1–30.

Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK, Hartl FU. 2000. Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci U S A* 97: 7841–7846.

Nardai G, Csermely P, Söti C. 2002. Chaperone function and chaperone overload in the aged. A preliminary analysis. *Expt Gerontol* 37: 1257–1262.

Njemini R, Abeele MV, Demanet C, Lambert M, Vandebosch S, Mets T. 2002. Age-related decrease in the inducibility of heat-shock protein 70 in human peripheral blood mononuclear cells. *J Clin Immunol* 22: 195–205.

Parsell DA, Lindquist S. 1994. Heat shock proteins and stress tolerance. In: *The Biology of Heat Shock Proteins and Molecular Chaperones*, ed Morimoto RI, Tissières A, Georgopoulos C. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 457–494.

Pawelec G, Adibzadeh M, Solanaa R, Beckman I. 1997. The T cell in the aging individuals. *Mech Ageing Dev* 93: 35–45.

Pittet JF, Lee H, Morabito D, Howard MB, Welch WJ, Mackersie RC. 2002. Serum levels of Hsp 72 measured early after trauma correlate with survival. *J Trauma* 52: 611–617.

Plumier C, Krueger AM, Currie RW, Kontoyiannis D, Kollias G, Pagoulatos GN. 1997. Transgenic mice expressing the human inducible Hsp70 have hippocampal neurons resistant to ischemic injury. *Cell Stress Chaperones* 2: 162–167.

Plumier C, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN. 1995. Transgenic mice expression of the human heat shock protein 70 have improved post-ischemic myocardial recovery. *J Clin Invest* 95: 1854–1860.

Pockley AG, Bulmer J, Hanks BM, Wright BH. 1999. Identification of human heat shock protein 60 (Hsp60) and anti-Hsp60 antibodies in the peripheral circulation of normal individuals. *Cell Stress Chaperones* 4: 29–35.

Pockley AG, Shepherd J, Corton J. 1998. Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. *Immunol Investig* 27: 367–377.

Prohaszka Z, Singh M, Nagy K, Kiss E, Lakos G, Duba J, Fust G. 2002. Heat shock protein 70 is a potent activator of the human complement system. *Cell Stress Chaperones* 7: 17–22.

Rao DV, Boyle GM, Parsons PG, Watson K, Jones GL. 2003. Influence of aging, heat shocktreatment and in vivo total antioxidant status on gene-expression profile and protein synthesis in human peripheral lymphocytes. *Mech Ageing Dev* 124: 55–69.

Rao DV, Watson K, Jones GL. 1999. Age-related attenuation in the expression of the major heat shock proteins in human peripheral lymphocytes. *Mech Ageing Dev* 107: 105–118.

Rea IM, McNerlan S, Pockley AG. 2001. Serum heat shock protein and anti-heat shock protein antibody levels in aging. *Exp Gerontol* 36: 341–352.

Sherman MY, Goldberg AL. 2001. Cellular defense against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* 29: 15–32.

Söti C, Csermely P. 2002. Chaperones come of age. *Cell Stress Chaperones* 7: 186–190.

Tanguay RM, Wu Y, Khandjian EW. 1993. Tissue-specific expression of heat shock stress proteins of the mouse in the absence of stress. *Dev Genet* 14: 112–118.

Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA. 2001. Exercise increases serum Hsp72 in humans. *Cell Stress Chaperones* 6: 386–393.

Wang ZZ, Wang CL, Wu T, Pan HN, Wang SK, Jiang JD. 2001. Autoantibody response to heat shock protein 70 in patients with heat stroke. *Am J Med* 111: 654–657.

Welch WJ. 1992. Mammalian stress response: cell physiology, structure/function of stress proteins and implications for medicine and disease. *Physiol Rev* 72: 1063–1081.

Wright BH, Corton JM, El-Nahas AM, Wood RF, Pockley AG. 2000. Elevated levels of circulating heat shock protein (Hsp70) in peripheral and renal vascular disease. *Heart Vessels* 15: 18–22.

Wu T, Chen S, Sun Y, et al. 2001a. Presence of antibody against the inducible Hsp71 in patients with acute heat-induced illness. *Cell Stress Chaperones* 6: 113–120.

Wu T, Ma J, Chen S, et al. 2001b. Association of plasma antibodies against the inducible Hsp70 with hypertension and harsh working conditions. *Cell Stress Chaperones* 6: 394–401.

Wu T, Yuan Y, Wu Y, He H, Zhang G, Tanguay RM. 1998. Presence of antibodies to heat stress proteins in workers exposed to benzene and in patients with benzene poisoning. *Cell Stress Chaperones* 3: 161–167.

Xiao C, Chen S, Li J, et al. 2002. Association of HSP70 and genotoxic damage in lymphocytes of workers exposed to coke-oven emission. *Cell Stress Chaperones* 7: 396–402.

Xiao C, Wu T, Ren A, et al. 2003. Basal and inducible levels of Hsp70 in patients with acute heat-induced illness induced during training. *Cell Stress Chaperones* 8: 86–92.

Xu Q, Kiechl S, Mayr M, Metzler B, Egger G, Oberhollenzer F, Willeit J, Wick G. 1999. Association of serum antibodies to heat shock protein 65 with carotid atherosclerosis: clinical significance determined in a follow-up study. *Circulation* 100: 1169–1174.

Zugel U, Kaufmann SHE. 1999. Role of heat shock proteins in protection from and pathogenesis of infectious diseases. *Clin Microbiol Rev* 12: 19–39.